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Ethanol from Lignocellulose:
The DOE/SERI Biochemical Conversion/Alcohol Fuels Research Program

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Abstract

Ethanol, a high octane liquid fuel compatible with today's transportation system, can be produced by biological processes from lignocellulosic feedstocks. Biomass is an attractive feedstock for ethanol production because it is available in large quantities at low cost. The chief advantage of biological processes is that they have the potential to convert wood to liquid fuels with an efficiency approaching 100%. However, in order to achieve this, each fraction of the lignocellulosic feedstock must be processed separately. The Biochemical Conversion/Alcohol Fuels Research Program managed by the Solar Energy Research Institute (SERI) for the Department of Energy Biofuels and Municipal Waste Technology Division carries out a program of research and development with the goals of developing processes for converting lignocellulosic materials to ethanol and other fuels in an efficient and cost-effective manner, and facilitating the adoption of these processes by industry.

Cellulose (a crystalline polymer of glucose), the structural core, comprises 40%-50% of the feedstock. It is difficult to hydrolyze (break down) into glucose, but once formed, the glucose is readily fermented to ethanol. Cellulose can be hydrolyzed with either acid or enzymatic catalysts. In acid hydrolysis, the same acids that catalyze the hydrolysis of cellulose to ethanol also degrade the glucose product. Thus the chief challenge is to develop systems that minimize degradation. Enzymatic processes are potentially much more efficient because enzymes catalyze only the hydrolysis reaction. However, these processes are much newer and less developed. Research is required to develop methods to make lignocellulose more digestible, to produce more active enzymes at lower cost, and to develop methods of hydrolysis where enzyme action is not inhibited by reaction end products. Approximately 30% of the feedstock is hemicellulose, which is largely made of xylan (a polymer of xylose). Xylan is easily hydrolyzed, but it is difficult to ferment to ethanol. Several potential methods of fermentation have been identified, but it is not yet clear which is the most promising. Lignin, the final major component, is a phenolic polymer that cannot be fermented to ethanol. However, it can be broken down to phenolic monomers, hydrotreated, and reacted with methanol to produce methyl aryl ethers, a high-octane gasoline additive.

The Need for Liquid Fuels

The economy of the United States, and indeed our entire modern way of life, is made possible by an infrastructure of automobile, truck, train, and airplane transportation unmatched in the world. For the foreseeable future, this system is dependent on the use of liquid fuels such as gasoline, diesel fuel, and jet fuel. Liquid fuels are indispensable because they pack a large amount of easily usable energy into a very small volume. No other power source (compressed gases, hydrogen, advanced batteries) comes close to the convenience of liquid fuels.

Almost all of our liquid fuels are currently made from petroleum, which today accounts for 36% of our total energy usage. Twenty years ago the United States was essentially self-sufficient in oil and was the world's largest producer (Figure 1). In the past two decades consumption has grown, yet production has decreased to the point where we now import approximately 30% of our oil. By the end of the century, imports are expected to account for almost 60% of our total oil consumption (Figure 2). Further, the United States accounts for 36% of the world's oil consumption, and the developed nations combined (United States, Western Europe, and Japan) account for 75%. At the same time, the United States possesses only 5% of the world's oil reserves while OPEC controls more than 75% (Conoco 1986). Therefore, OPEC will again come to dominate the world energy markets. Although it is difficult to say exactly what this means for energy prices, inevitably the power to control them will shift back to the OPEC oil-producing nations. This shift in control will again be followed by sharp increases in oil prices. As we have

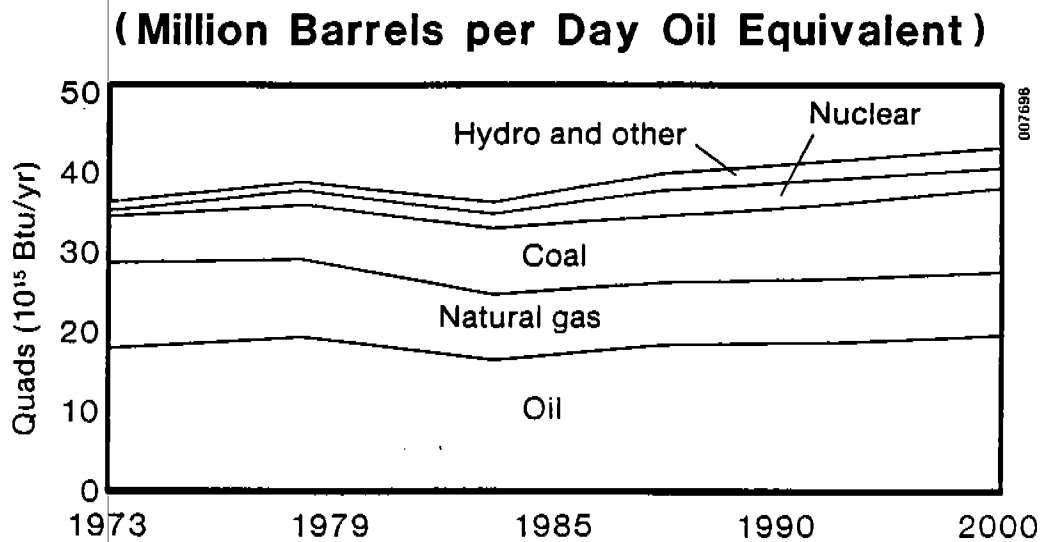


Figure 1. U.S. Energy Supply

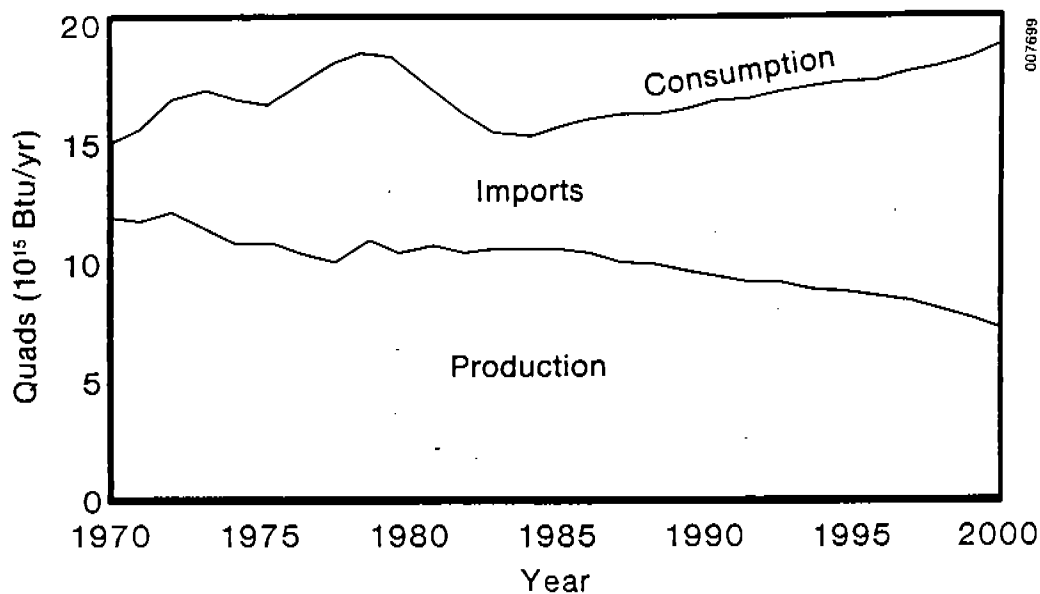


Figure 2. U.S. Oil Consumption and Production

seen before, this leads to an enormous drain on the U.S. balance of payments, significantly reduced economic growth and increased inflation, and a serious risk to our security as a nation. It is in our best interest to develop alternative liquid-fuel sources over which we have control, to counter OPEC's increase in dominance of world oil supplies.

Fuel Ethanol

More than 60% of U.S. oil consumption is used for transportation, with the bulk as motor gasoline (Figure 3); thus our primary need is for a gasoline substitute or extender. It is important that this fuel can be used interchangeably with gasoline so that the fuel can be used in the existing transportation fleet.

Ethanol can be mixed with gasoline at concentrations of up to 20%, and the resulting blend can be used interchangeably with standard gasoline without modifying current gasoline engines appreciably. Ethanol also has a very high octane value when blended with gasoline. In fact, in many parts of the country, the majority of the premium unleaded gasoline sold is an ethanol/gasoline blend. These mixtures are often cleaner burning fuels than gasoline itself, especially at high altitudes, where the oxygen in the ethanol helps the fuel to burn more cleanly. Although a gallon of ethanol contains only two-thirds the energy in a gallon of gasoline, vehicles run on 10%/90% ethanol/gasoline mixtures appear to get essentially the same number of miles per gallon as those run on 100% gasoline. Perhaps the most convincing statistic showing that ethanol gasoline blends are compatible with the U.S. transportation system is that in 1986 approximately 10% of the gasoline sold in this country was an ethanol blend.

Ethanol Production

People have known how to produce ethanol for at least 8000 years. The ability of yeast to make alcohol (ethanol) in the form of beer was known to the Sumerians and Babylonians before 6000 BC. Another ancient product of fermentation, wine, is referred to in

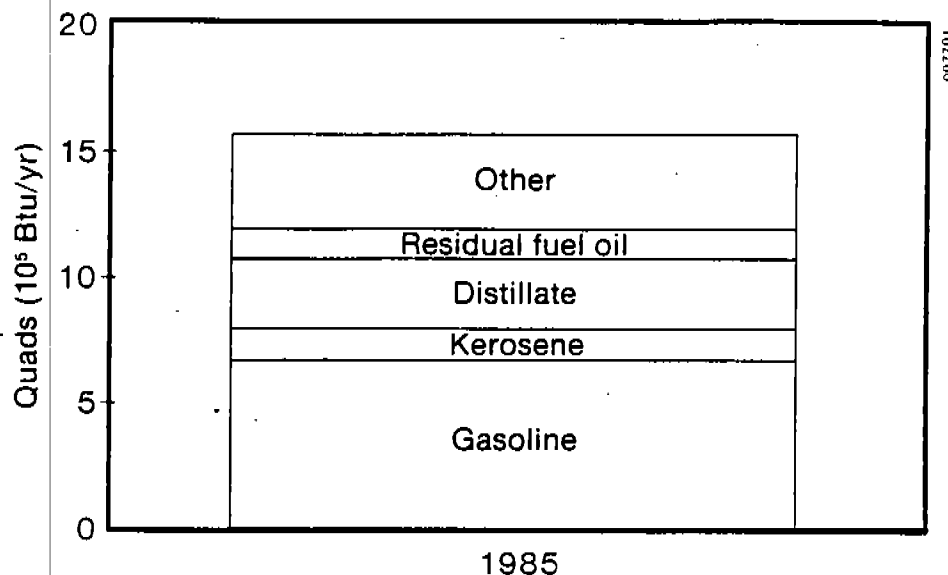


Figure 3. U.S. Oil Product Demand

the book of Genesis. By the 14th century, the distillation of alcohol from grain was common in many parts of the world.

In the absence of air, yeast converts sugar into ethanol and carbon dioxide. Given a concentrated sugar solution, yeast can produce an ethanol concentration of from 10%-12%. From this solution, the ethanol is concentrated to essentially 100% purity by distillation.

Ethanol can be made from a number of renewable feedstocks, including sugar crops such as sugar cane, starch-containing grains such as corn, lignocellulosic materials including corn stover, other crop residues, and wood. The easiest of these to process are the sugar crops. For example, in making fuel ethanol from cane sugar, the sugar cane (containing 20%-30% sugar) is first chopped into short pieces and crushed. The sugar is then easily washed from the cane with hot water, fermented by yeast to produce a 10% ethanol stream, and concentrated by distillation. The economics of this process are dominated by the cost of sugar. At sugar prices prevailing in the tropical developing countries (6¢-7¢/lb), the feedstock contributes approximately 80¢/gal to the cost of ethanol. In the United States, where sugar is more expensive because of higher labor costs and a less advantageous climate, the 20¢-30¢/lb of sugar would contribute up to \$4/gal of ethanol. Brazil currently makes 3 billion gallons a year of ethanol from sugar cane. In Brazil all cars are run on either a 20% mixture of ethanol with gasoline or pure ethanol. However, in the United States, because of higher sugar prices, virtually no ethanol is made from sugar cane.

In this country most fermentation ethanol is made from corn. Corn and other grains are roughly 80% starch, which is a polymer of glucose. Enzymes hydrolyze the starch chains into individual sugar molecules that are then fermented in the conventional fashion to produce ethanol. At the current depressed corn prices (\$1.50-\$2.00/bushel), the feedstock cost contributes 30¢/gal of ethanol (after significant credits for high-protein animal feed by-products). Virtually all of the U.S. fuel ethanol production of almost one billion gallons per year is made from corn. It is estimated that an additional 5-6 billion gallons per year of ethanol could be made with surplus corn.

Ethanol produced from corn is competitive in the United States even at today's depressed oil prices because of the current depressed farm economy, which means low corn prices, and the availability of federal tax credits that total 60¢/gal of ethanol produced. If the ethanol energy industry were to flourish enough, the current corn surplus would be used up and corn prices would rise, which would raise the price of ethanol and eventually end the industry growth. To produce even greater amounts of ethanol, a more plentiful and less expensive source of sugar is needed.

Lignocellulosic materials are such a large and inexpensive resource that existing supplies could support a sustainable production of approximately 10 quads (1 quad = 10 Btu/yr). This is roughly equivalent to two-thirds of our transportation fuel usage. Thus, ethanol derived from lignocellulosic biomass has the potential to produce liquid transportation fuels on the same scale as the total U.S. consumption. Lignocellulosic materials are inexpensive because they have no value as a food. However, up to three-quarters of the biomass could be converted into fermentable sugars. An average biomass price of \$40/dry ton amounts to approximately 3¢/lb of sugar or a contribution to the feedstock costs of as low as 35¢/gal.

Ethanol Production Processes

While lignocellulose is inexpensive because it cannot be digested and therefore does not compete as a food, its inability to be digested makes it difficult to convert to fermentable sugars. Furthermore, lignocellulose is a complex structure with three major components, each of which must be processed separately to make the best use of the high efficiencies inherent in biological processes. Thus, we have an inexpensive, but difficult to process, feedstock. Our challenge is to develop efficient and inexpensive processes that can use the entire feedstock.

The largest fraction of lignocellulose (approximately 50% of the entire mass) is crystalline cellulose. This component consists of long chains of glucose molecules that are difficult to digest and convert to simple fermentable sugars because of their crystalline packing. However, once the sugars are produced they are easy to ferment. The second fraction is hemicellulose (25%), which consists of polymers of the five-carbon sugar xylose. Because this fraction is not crystalline it is relatively easy to hydrolyze to simple sugars; however, the xylose sugars are difficult to ferment to ethanol. The final fraction is lignin (25%). Lignin is not a sugar polymer but a phenolic polymer and therefore cannot be fermented to produce ethanol. It must be converted thermochemically into a liquid fuel.

A general schematic for the conversion of lignocellulose to ethanol is shown in Figure 4. The lignocellulose is brought to the plant and pretreated to separate the xylose and sometimes the lignin from the crystalline cellulose. The xylose can then be fermented to ethanol and the lignin processed to produce other liquid fuels. The crystalline cellulose remains behind as a solid after the pretreatment and is sent to either an acid or an enzymatic hydrolysis process that breaks the cellulose down into glucose. The glucose is then fermented to ethanol and combined with the ethanol from the xylose fermentation. This dilute beer is then concentrated to fuel-grade ethanol by distillation.

Crystalline cellulose, the largest and most difficult fraction, is hydrolyzed by the acid and enzymatic processes. Acid processing is a relatively near-term option that has been studied for many years. However, it is quite indiscriminate, and attacks and degrades product sugars before they can be fermented. Thus the central challenge of acid hydrolysis is to devise methods of hydrolyzing the crystalline cellulose to glucose without suffering undue degradation of the product sugars.

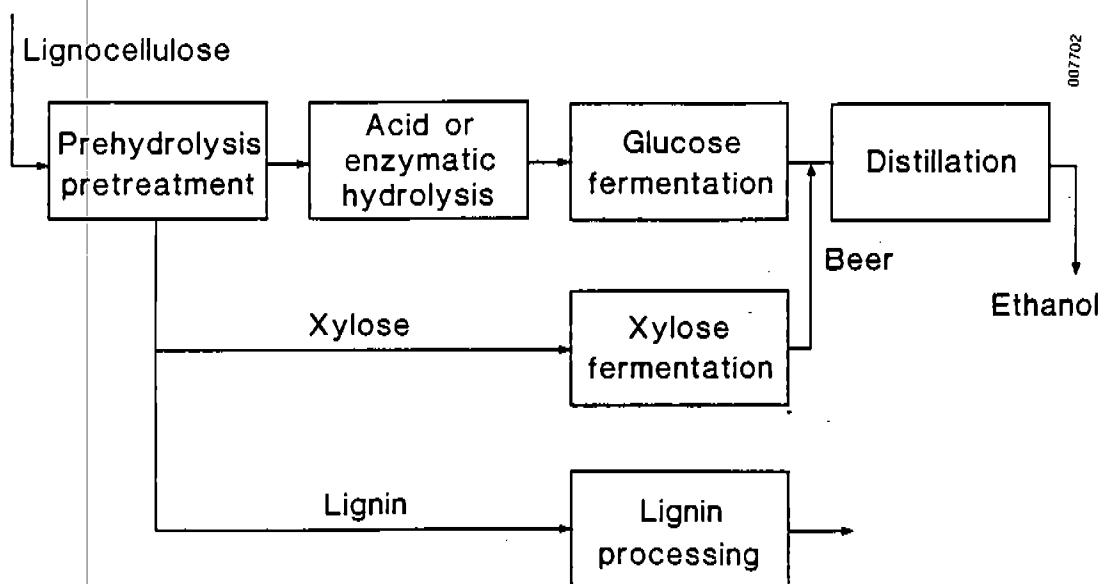


Figure 4. Conversion of Lignocellulose to Ethanol

Enzymes are biological catalysts that generally are designed to do one job well, but to do one job only. For this reason, the enzymes that catalyze the hydrolysis of cellulose to sugar do not break down the sugars. Thus enzymatic processes are capable of yields approaching 100%. Enzymatic hydrolysis processes have been under development for only 5 years or so. While improvements have been made in enzymatic technology, more are both possible and necessary. The important research issues include understanding the processes necessary to render the crystalline cellulose easily digestible, understanding and improving the basic mechanisms in the hydrolysis step, and developing better and less expensive enzymes.

The hemicellulose fraction is composed primarily of xylan, which is easy to convert to the simple sugar xylose, but the xylose is difficult to ferment to ethanol. Ten years ago there were no practical systems for xylose fermentation. Today methods have been identified using new yeasts, fungi, bacteria, and processes combining enzymes and yeasts. Although none of these fermentations are yet ready for commercial use, considerable progress has been made.

Lignin, the third major component of lignocellulose, is a large, random phenolic polymer. In lignin processing, the large polymer is broken down to smaller fragments containing one or two phenolic rings. Extra oxygen and side chains are stripped from the molecules by catalytic methods, and the resulting phenol groups are reacted with methanol to produce methyl aryl ethers. Methyl aryl ethers are a high-value octane enhancers that can be blended with gasoline.

The following sections describe the state of the art in each of these major sections of the process and the current research efforts in the Biochemical Conversion/Alcohol Fuels Research Program in each of the major areas (cellulose hydrolysis, xylose fermentation, and lignin conversion). Table 1 lists the efforts that are now under way, including their location and the principal investigator.

Table 1. Structure of the Biochemical Conversion/Alcohol Fuels Research Program

| Project | Location | Principal Investigator |
|--|--|------------------------|
| <u>Acid Hydrolysis</u> | | |
| Plug Flow Dilute Acid Hydrolysis Reactor | SERI | A. Brennan |
| Fermentation of Dilute Acid Hydrolyzates | Tennessee Valley Authority | M. Beck |
| Progressing Batch Dilute Acid Hydrolysis Reactor | SERI | P. Bergeron |
| Packed Bed Hydrodynamics in the Progressing Batch | Brown University | J. Calo |
| <u>Hydrolysis Reactor</u> | | |
| Concentrated Sulfuric Acid Hydrolysis | TVA | W. Barrier |
| Acid Recycle on Polyamine Resins | Purdue University | G. Tsao |
| Acid Recovery by Solvent Extraction | University of Arkansas | J. Gaddy |
| Acid Recovery by Adsorption | University of Missouri-Rolla | O. Sitton |
| Dilute Acid Pretreatment | SERI | K. Grohmann |
| Transport of Acid Catalysts in Biomass Pretreatment | Auburn University | Y. Lee |
| Organosolv Pretreatment | SERI | H. Chum |
| Cell Wall Mechanism of Resistance to Enzymatic Attack | Texas A&M | |
| NMR Studies of Resistance to Enzymatic Hydrolysis | Colorado State University (CSU)/SERI | |
| Pretreatment and Enzyme Kinetic Interaction of Feedstock Properties and Conversion | Dartmouth College Oak Ridge National Laboratory (ORNL)/SERI | A. Converse |
| <u>Enzymatic Hydrolysis</u> | | |
| Simultaneous Saccharification and Fermentation (SSF) | SERI | C. Wyman |
| Modeling of Mixed Culture SSF Processes | University of Colorado/SERI | D. Kompala |
| Mixing Requirements in Enzymatic Hydrolysis | Colorado State University | V. Murphy |
| Continuous Enzymatic Hydrolysis Pretreatment of MSW for SSF | Texas Tech/SERI | S. Beck |
| Enzyme Crosslinking for Stability and Recycle | CADCO | F. Richerson |
| Stoichiometry of the Active Enzyme Complex | SERI | M. Himmel |
| | CSU/SERI | R. Ellis |

Table 1. Structure of the Biochemical Conversion/Alcohol Fuels Research Program (Concluded)

| Project | Location | Principal Investigator |
|---|-------------------------------|------------------------|
| Cellulase Synergism and Recycle Genetic Improvement of the CBH-Cellulase Enzyme | ORNL/SERI | J. Woodward |
| High Temperature <i>A. cellulolyticus</i> enzymes | Genencor/SERI | S. Shoemaker |
| Enzyme Production of Cellulose-Xylose Mixtures | SERI | M. Himmel |
| Enzyme Production in Fluidized-Bed Reactors | SERI | A. Mohagheghi |
| <i>T. reesei</i> Strain Improvement | CSU/SERI | R. Tengerdy |
| Regulation of Cellulase Synthesis | Lehigh University | B. Montenecourt |
| Enzyme Production in <i>A. cellulolyticus</i> | Rutgers University | D. Eveleigh |
| | CSU/SERI | J. Linden |
| <u>Xylose Fermentation</u> | | |
| Engineering Evaluation of Xylose Fermentation | SERI | N. Hinman |
| Simultaneous Isomerization and Fermentation of Xylose | SERI | S. Lastick |
| Overproduction of Xylose Isomerase in Genetically | Virginia Poly-Tech./SERI | M. Potts |
| Cloning of Xylulokinase for Improved Xylulose | Purdue University | N. Ho |
| Xylose Fermentation with Yeast | TVA | M.J. Beck |
| Fusarium Xylose Fermentations | Argonne National Laboratory | A. Antonopoulos |
| <u>Lignin Conversion</u> | | |
| Lignin Characterization | SERI | H. Chum |
| Methyl Aryl Ethers from Lignin | SERI | D. Johnson |
| Catalyst Development for Fuels from Lignin | Colorado School of Mines/SERI | S. Cowley |
| Engineering for Fuels from Lignin Fermentation | Colorado School of Mines/SERI | S. Baldwin |
| Engineered <i>E. coli</i> | | |

Acid Hydrolysis

Acid hydrolysis processes are relatively well understood and can largely make use of existing chemical process equipment. Most of these processes are now at the stage where integrated testing is required to establish their performance.

The economics of five major acid hydrolysis processes (at the current state of the technology) are shown in Table 2. The relative economics of the current state of the art and processes incorporating most of the important potential process improvements are shown in Figure 5 (Wright and Power 1986). In all cases, the single most important improvement in the advanced case is to incorporate a xylose fermentation process. The progressing batch reactor has the potential for higher yields than the plug flow reactor, but this potential has yet to be achieved in practice. The economics of the plug flow reactor are dominated by the value assigned to the furfural by-product. The concentrated acid processes all have high yields and low feedstock costs but suffer from the cost of acid consumption or acid recovery.

In dilute acid hydrolysis processes, sugar degradation and the consequent low yields are the main problems. The progressing batch reactor system uses several percolation reactors in series to achieve the benefits of countercurrent operation while retaining the simplicity and proven technology of the percolation reactor. This method quickly removes the sugars from the reaction zone, thus minimizing both sugar degradation and product dilution (Wright et al. 1987; Bergeron et al. 1986). Experiments are now under way at SERI to test this concept. The principal problem appears to be that flow through the bed of wood chips is not uniform, which leads to some of the sugars remaining in the reactor longer than is desirable. This causes increased sugar degradation. Therefore, Brown University is carrying out a study to better understand the flow regimes in such a reactor.

The high-temperature dilute acid plug flow reactor (originally developed at Dartmouth College and now being scaled up at SERI) has low yields (55% of the crystalline cellulose is converted to glucose) but can simultaneously convert the xylan fraction to furfural, a valuable chemical intermediate, at yields of up to 70%. The value assigned to the furfural greatly influences the process economics. Furfural production may provide a significant near-term advantage and highlights the importance of beneficially using all components of the feedstock. Operation at a solids concentration of approximately 20% is necessary for favorable economics, and is achieved by carrying out a prehydrolysis step before the main hydrolysis, thus solubilizing approximately 30% of the feedstock and rendering it more easily pumpable (Brennan et al. 1986). The keys to this process are the high by-product credit for furfural, and the achievement of high concentrations of fermentable sugars in the hydrolyzate. This project is no longer being supported by the program because the program has been focused on high yield processes to produce fuels.

However, the production of furfural is a valuable asset, and chemicals other than ethanol can be produced by this reactor. It is therefore undergoing continued development at SERI with funding from private industry.

Both dilute acid hydrolysis processes produce large amounts of sugar degradation products, which are inhibitory to the fermentation process. Therefore, treatment steps are necessary to remove these compounds from the hydrolyzate and prepare it for fermentation. Studies of such methods are being performed by the TVA.

In the concentrated sulfuric acid hydrolysis process the concentrated acid breaks down the crystalline structure of the cellulose, rendering it amorphous and readily hydrolyzable at conditions where sugar degradation is minimal and yields approach 100%. The

Table 2. Cost of Production Summary for Lignocellulose-to-Ethanol Facilities with Various Hydrolysis Processes (25,000,000 gal/yr, Mixed Hardwood at \$42/ton).

| | Cost of Production (¢/gal Ethanol) | | | | | |
|----------------------------|------------------------------------|--------------|-------------------|---|--------------|--------------|
| | Plug Flow | Plug Flow* | Progressing Batch | Concentrated H ₂ SO ₄ | Liquid HCl | Liquid HF |
| Raw materials | | | | | | |
| Wood | 104.6 | 104.6 | 77.5 | 68.2 | 69.9 | 62.1 |
| Acid | 6.5 | 6.5 | 6.5 | 17.4 | 11.9 | 39.9 |
| Lime | 3.5 | 3.5 | 3.5 | 9.4 | 1.6 | 0.7 |
| Chemicals | 3.5 | 3.5 | 2.6 | 2.3 | 2.3 | 2.1 |
| Utilities | | | | | | |
| Water | 0.9 | 0.9 | 1.1 | 1.3 | 0.9 | 0.5 |
| Steam | 0.0 | 0.0 | 0.0 | 0.0 | 35.2 | 2.7 |
| Labor | 7.2 | 7.1 | 6.0 | 7.6 | 7.4 | 5.9 |
| Overhead & maintenance | 34.0 | 33.7 | 28.6 | 35.9 | 35.3 | 27.9 |
| Byproducts | | | | | | |
| Furfural | -70.5 | 0.0 | -3.2 | 0.0 | 0.0 | 0.0 |
| Electricity | -20.8 | -31.0 | -23.8 | -21.8 | 22.3 | -17.6 |
| Annual operating cost | <u>68.8</u> | <u>128.8</u> | <u>98.8</u> | <u>104.7</u> | <u>139.6</u> | <u>126.1</u> |
| Capital charges (FCR=0.13) | <u>60.0</u> | <u>59.4</u> | <u>50.3</u> | <u>63.2</u> | <u>62.1</u> | <u>49.2</u> |
| Ethanol selling price | 128.8 | 188.2 | 149.1 | 182.4 | 201.8 | 175.2 |

*Furfural concentrated and burned for energy production.

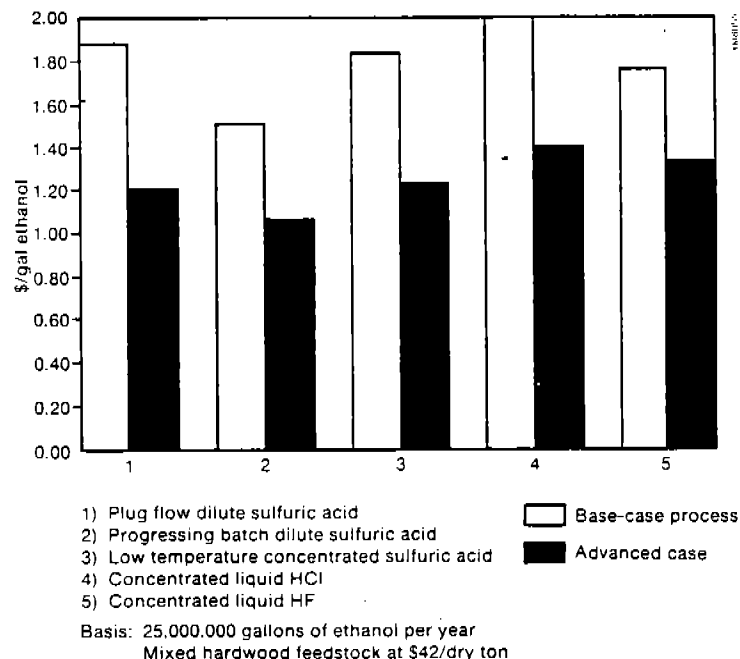


Figure 5. Comparison of Acid Hydrolysis Processes for the Production of Ethanol

major barrier to economic success is the high cost of sulfuric acid consumption. Research to improve the process itself is being carried out at TVA. Efforts are focused on reducing acid consumption, improving the processing equipment, waste treatment, and obtaining high conversion yields on a variety of feedstocks. Acid recycle is the key to improved process economics in concentrated acid hydrolysis. Purdue University has studied a method using polyamine resins to separate acids and sugars, while the University of Arkansas has studied solvent extraction systems, and the University of Missouri has studied adsorption processes.

Enzymatic Hydrolysis

All enzymatic hydrolysis processes consist of four major steps that may be combined in a variety of ways: pretreatment, enzyme production, hydrolysis, and fermentation. Although shielding of the cellulosic surface by lignin, crystallinity, and the inaccessibility of the cellulose to the enzymes have all been suggested barriers to enzymatic attack (Fan et al. 1982), it now appears that the key to increasing the digestibility of lignocellulose lies in increasing the cellulose surface area that is accessible to the enzymes. The internal surface area of wood is large, but only 20% is accessible to large enzymes such as cellulase (MW of 20-60,000 Daltons, major and minor dimensions of 300 x 30 angstroms; Cowling and Kirk 1976). Through prehydrolysis the hemicellulose fraction can be removed, thus enlarging the pore size and opening the structure to attack by the enzymes (Grethlein et al. 1984). Further, the degree of digestibility is almost directly proportional to the fraction of the xylan removed (Grohmann et al. 1985). Thus, all the major pretreatment options, dilute acid, steam explosion (Brownell and Saddler 1984), and organosolv processes (Holtzapple and Humphrey 1984), are acid-catalyzed removal of hemicellulose and in the case of organosolv, hemicellulose, and lignin. Pretreatment research includes dilute acid pretreatment, organosolv pretreatment, and efforts to understand the interaction between pretreatment and hydrolysis. Steam explosion is not studied because it degrades a substantial amount of the hemicellulose into furan compounds.

Dilute acid pretreatment is being investigated because it is simple, effectively increases the digestibility of the biomass, and converts approximately 80% of the xylan into xylose for fermentation. Research on this process at SERI has shown that the material produced in the pretreatment process must be smaller than one sixteenth of an inch in order for enzymatic hydrolysis to proceed at a rate that is not limited by diffusion of the enzyme into the interior of the particle. Engineering on the dilute acid pretreatment system is being carried out at Auburn University. Also, a two-stage process in which dilute acid removes the hemicellulose, and dilute sodium hydroxide next removes the lignin is being investigated. Removal of the lignin simplifies the subsequent hydrolysis. Perhaps more importantly, the base treatment improves the rate of enzymatic hydrolysis by almost a factor of seven. Understanding this effect and how to take advantage of it could greatly improve hydrolysis processes. However, the base treatment also degrades part of the cellulose into organic acids, reducing the yield. This must be overcome for this process to be useful. The organosolv pretreatment simultaneously removes hemicellulose and lignin. The lignin is removed in a reactive form that is amenable to further processing. Also, removal of the lignin greatly simplifies the subsequent enzymatic hydrolysis. However, these advantages come at the cost of increased capital and operating costs. Although accessibility of the substrate to the enzyme has been shown to be the most critical factor in enhancing enzymatic digestibility, there are several more subtle effects involving the structure of the lignocellulosic matrix. These phenomena and their relationship to enzymatic hydrolysis are being studied under new subcontracts to Texas A&M and Colorado State University. These projects will selectively modify or remove constituents of the lignocellulosic matrix (acetyl groups, xylan, specific lignin bonds) to determine their individual effects on hydrolysis. Research at Dartmouth College also seeks improved understanding of this structure-digestibility relationship, and will develop a model linking these effects to hydrolysis kinetics.

A new initiative is the integration of the feedstock production and the conversion process. While a processing facility using existing forest resources or agricultural residues will have to process whatever is available, the use of woody or herbaceous feedstocks grown specifically for use in a conversion facility opens new opportunities. These feedstocks can be tailored for improved performance and value in conversion. For example, the carbohydrate content of the biomass may be enhanced, the digestibility by enzymes increased, or the ease of delignification improved by modifying the lignin composition. In the coming year, studies will be performed on the enzymatic hydrolysis of bark. Bark constitutes a greater fraction of short rotation wood grown specifically for conversion, but is high in extractives, a natural defense against enzymatic attack. Further, screening studies will be carried out on short rotation forestry feedstocks and lignocellulosic herbaceous energy crops to identify both potential problems and valuable traits that could be improved by breeding and selection.

The hydrolysis of cellulose is carried out by a complex of enzymes that have three different modes of action, as shown in Figure 6 (Wood 1985). First, the endo-glucanase absorbs on the surface of the solid cellulose and attacks the interior of the polymer chain, splitting it and exposing two new chain ends. Next, exo- β -glucanases remove cellobiose units (two linked glucose units) from the non-reducing end of the cellulose chain. The cellobiose produced by this reaction can accumulate in solution and strongly inhibit the activity of the exo-glucanase. Finally, in a liquid-phase reaction, β -glucosidase splits the cellobiose units into glucose. Similarly, the accumulation of glucose can inhibit the action of β -glucosidase, causing a buildup of cellobiose, which again inhibits the exo-glucanase activity. Thus, the successful production of glucose (the desired feedstock for ethanol production) can cause severe end-product inhibition, which can greatly limit concentration, yield, or reaction rate.

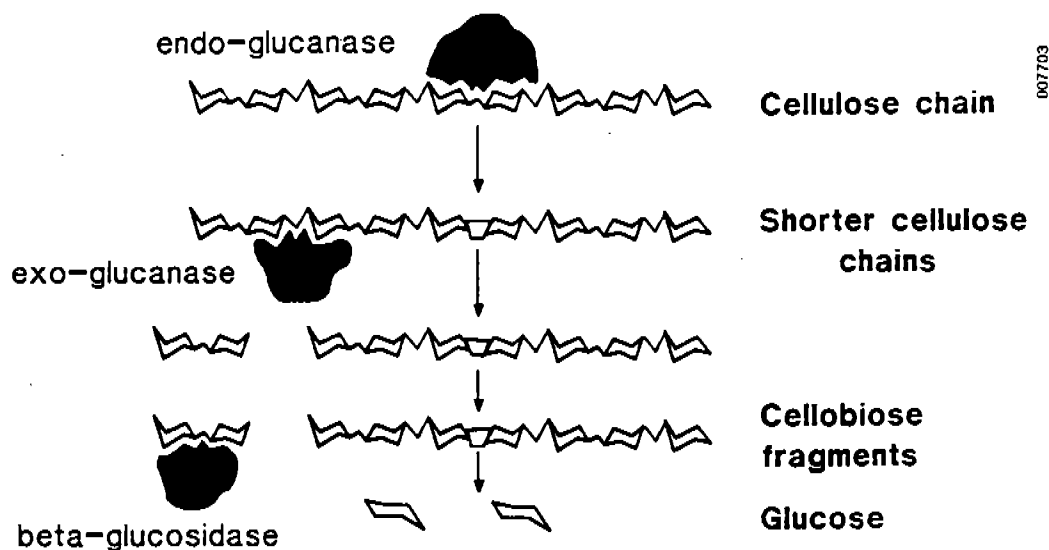


Figure 6. Mechanism of Enzymatic Hydrolysis and SSF

The major focus of the Biochemical Conversion/Alcohol Fuels Research Program and SERI in-house research is the SSF process (Wyman, Spindler, and Grohmann 1987; Wright, Wyman, and Grohmann 1987). To understand the rationale for SSF processes, it is useful to compare them to the separate hydrolysis and fermentation (SHF) (Wright and Power 1986). A cost breakdown is shown in Figure 7. The total cost of ethanol production is \$2.66/gal, with \$0.65/gal (25%) of the cost contributed by enzyme production. Approximately \$0.40/gal (15%) is by the cellulose fraction of the feedstock that is converted to ethanol, and an additional \$0.40/gal (15%) is attributable to the hydrolysis reactor section. The extremely high cost of enzyme production arises in part from the low rate of enzyme production caused by the use of an insoluble cellulose carbon source.

However, a much more important cause is the high consumption of cellulase caused by the end-product inhibition by cellobiose and glucose. The higher the final glucose concentration, the higher the loading of a given cellulase needed to achieve any given yield. Similarly, because the reaction is slowed or stopped by the presence of glucose and cellobiose, the hydrolysis is essentially halted before the reaction can proceed to completion. The optimal point for SHF is an enzyme loading of 20 IU/g substrate (33 IU/g cellulose), and a final glucose yield and concentration of 73% and 4.5%, respectively. Thus, end-product inhibition is in large part responsible for the limitations in yield, product concentration, reaction rate, and high enzyme loading that give SHF such a high cost of production.

One means of alleviating this problem is to use cellulase preparations that have higher β -glucosidase activities. These newer enzyme preparations (such as Genencor 150L) are less inhibited by glucose and also remove cellobiose more efficiently, allowing the reaction to proceed more quickly to higher yields and glucose concentration (Wyman et al. 1986). The improvement from using such an enzyme preparation is shown in Figure 8.

Even more improvement can be made by continuously removing the glucose through fermentation (SSF). Use of this process with Genencor 150L enzyme and *S. cerevisiae*

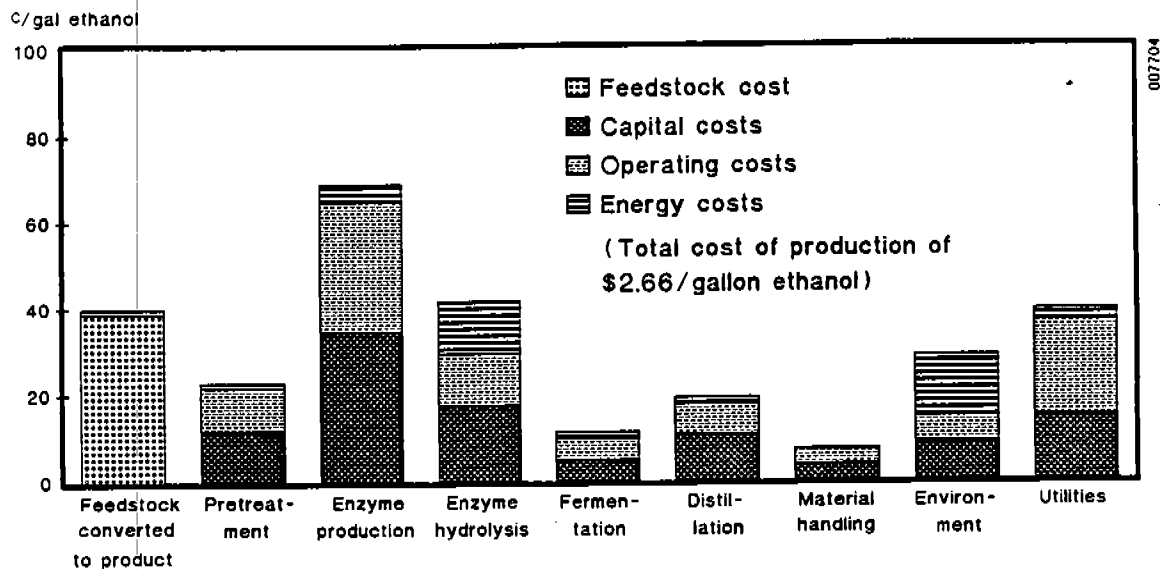


Figure 7. Breakdown of Ethanol Production Costs by Process Area for SHF Process

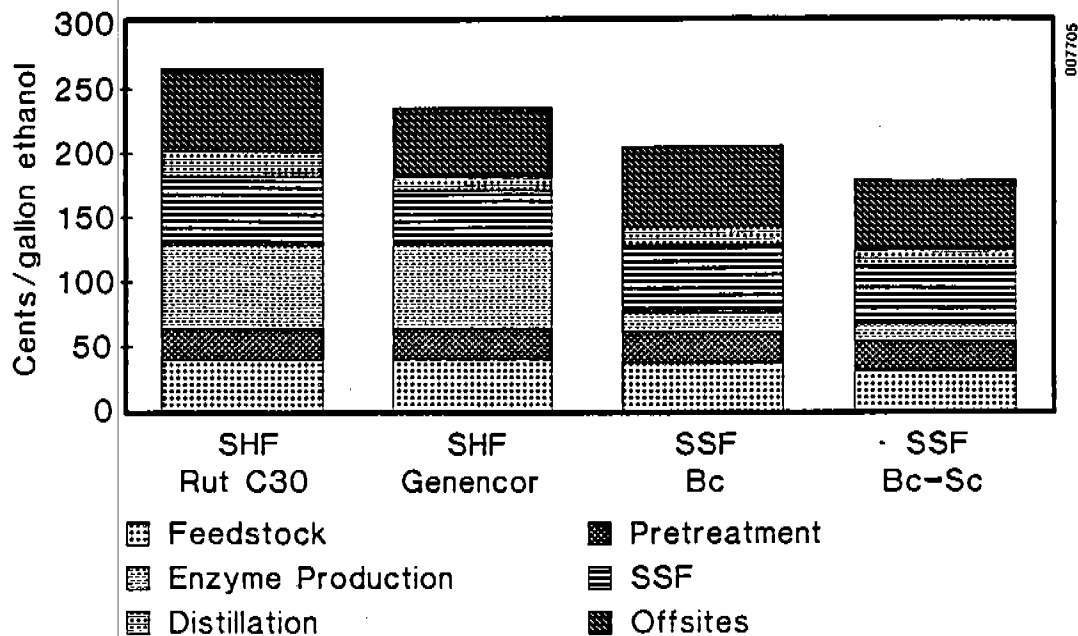


Figure 8. Improvements in Enzymatic Hydrolysis by Improved Enzymes and SSF Processes

(generally not considered one of the best single yeasts for SSF) further improves the performance. With the enzyme loading reduced by almost a factor of five to 7 IU/g cellulose, the hydrolysis yield remains at 73% and the ethanol concentration is increased to 3.7% (equivalent to a glucose concentration of 8.1%, roughly twice that of SHF). This further reduces the cost of ethanol production to \$2.06/gal (Figure 9). *C. brassicae* (Takagi et al. 1977), generally considered the organism of choice, gives a yield of 79% under similar conditions, resulting in an ethanol selling price of \$1.94/gal (Figures 8 and 9).

Since cellobiose is an even greater inhibitor than glucose, cellobiose-fermenting yeasts offer potential for further improvement. Using *B. clausenii* (a cellobiose-fermenting yeast) alone increased the yield to 83%. However, using a mixed culture of *S. cerevisiae* and *B. clausenii* further increased the yield to 88%. The increased performance comes from the fact that the *B. clausenii* cell density increases quickly early in the SSF when glucose and cellobiose are being produced rapidly. Thus, the *B. clausenii* is active early and removes the cellobiose inhibition when it is most important, providing high initial rates. However, it is not a particularly robust yeast and loses viability later in the fermentation when the rates of glucose and cellobiose production drop. Here, the much more robust and ethanol-tolerant *S. cerevisiae* remains viable and allows the reaction to proceed essentially to completion without end-product inhibition (Wyman et al. 1986). The mixed culture produces yields of 88% and ethanol concentrations of 4.5% from 10% cellulose, resulting in a predicted selling price of \$1.78/gal (Wright et al. 1987). The achievement of these levels of performance in an SSF process is one of the most important achievements of the program in the past year.

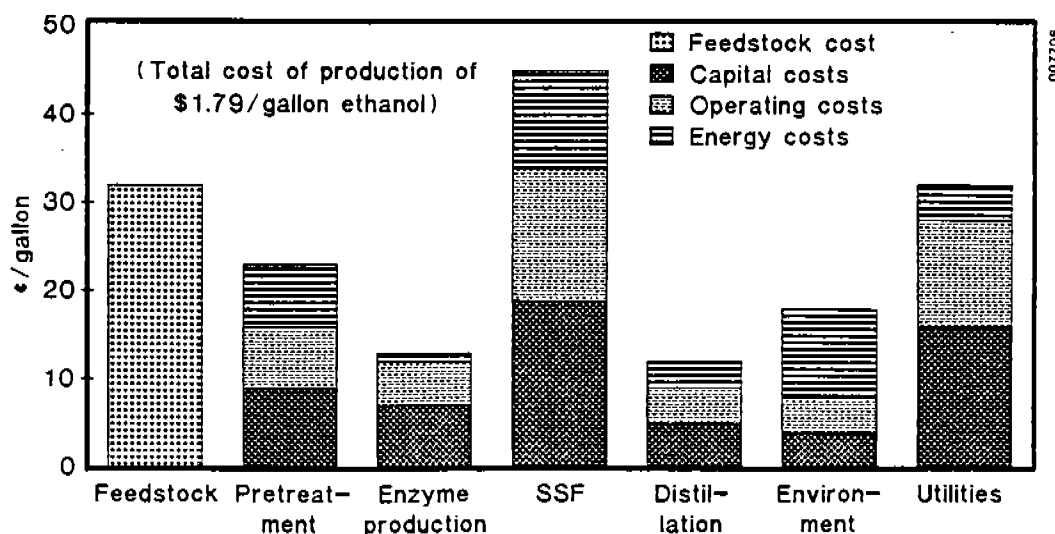


Figure 9. Breakdown of Ethanol Production Costs by Process Area for SSF Process

Supporting SERI's research on SSF performance is a project at the University of Colorado that is developing mathematical models of multiple substrate utilization by *B. clausenii*. Such models should point the way to improved operating strategies. Another process investigating operating strategies is being carried out at Texas Tech, where alternative designs for continuous SSF are being evaluated.

The high power required to maintain a viscous suspension of cellulose, lignin, and yeast has always plagued enzymatic hydrolysis. Recent CSU research is finding that it may not be necessary to suspend the solids, but only to prevent sugars from building up in some parts of the reactor while others are sugar free. If this is true, power requirements may be much less than previously assumed.

Municipal solid waste (MSW) is a promising feedstock for conversion to ethanol because it is readily available at a negative cost. Pretreatments for MSW have not previously been studied on a scale that will provide meaningful operating data. CADCO, a joint project of United Biofuels, Foster-Wheeler, Raphael Katzen Associates, Riddick Engineering, and the University of Arkansas, is investigating the relationship between pretreatment and SSF using pilot or commercial scale equipment.

The enzymes that catalyze the hydrolysis reaction are the heart of the SSF process, but many fundamental questions remain about their mechanism of action. Also, much of the improvement over the past decade is directly traceable to either the improvement of enzyme preparations, or improvements in our use of the enzyme, which grew directly out of better understanding of their mechanisms of action. Thus, improving our understanding of enzymology is an important component of the program.

Even with the low enzyme use of the SSF process, production of enzymes is expensive and requires use of carbohydrates that could be converted into ethanol. Thus, it would be useful to recycle the enzymes. Both the endo and exo components of cellulase have a high affinity for solid cellulose and may be recovered from the hydrolyzate solution by contacting the hydrolyzate with the fresh feed to the reactor. However, because the β -glucosidase component operates on the soluble sugar cellobiose, it has no affinity for cellulose, and is not recovered in the same process. Therefore, research is being carried out to link the cellobiose to one of the other enzymes so it can be recycled simultaneously. Similar cross-linking technology is being investigated to stabilize the β -glucosidase against thermal degradation.

Cellulose recycle by absorption on ion exchange resins is being investigated in a joint project between ORNL and SERI. Early investigations in this project have focused on measuring the activities of individual components of the enzyme mixture, and have yielded significant data on the interactions of the various components. A new project at Colorado State University will use monoclonal antibodies to attach colloidal gold to the exo and endo enzymes to determine if there is a physical association between these two components at the site of hydrolysis.

Researchers at Genencor will use genetic engineering techniques to better understand the structure of the active site of the exo cellulase enzyme cellobiohydrolase II. They will then use such genetic techniques to modify the structure of the enzyme in an attempt to improve its activity. Although earlier investigators improved the activity of cellulase enzyme preparations, most of this improvement has occurred because of changes in the ratios of the various components. This is the first attempt to make specific and defined changes in the structure of a specific enzyme in mixture. Any improvements coming out of this research can be quickly commercialized because Genencor is the major U.S. producer of cellulase in a market where the primary competitors are European and Japanese.

Operation of hydrolysis processes at higher temperatures could improve reaction rates. SERI researchers have isolated a high temperature aerobic bacteria (*Acidothermus cellulolyticus*) that produces a cellulase system more stable than any discovered to date. This may prove valuable in its own right, or provide clues to the mechanisms of thermal stability that may be applicable to other cellulases. CSU is developing methods to improve production of enzymes with this organism.

Cellulase enzymes are efficiently produced by the filamentous fungi *T. reesei* (Mandels 1981). Traditional methods of production use solid cellulose as both the inducer and the carbon source for enzyme production and growth. Although productivities of up to 150 IU/L-h have been reported from such methods (McLean and Podrutzny 1985), the productivities are limited by the low rate of hydrolysis (and hence low rate of growth) of the cellulose on the insoluble substrate. This low production rate makes enzyme production costs an important component of the overall processing cost. One promising alternative is to identify fungal mutants that produce enzymes while growing on soluble carbon sources such as lactose (Montenecourt 1983). However, lactose may not be available in the volumes required to produce enzymes for a major fuel industry. One method of improving production rate is to decouple growth and enzyme production. SERI has investigated a procedure where the fungi are grown rapidly on the soluble sugar xylose, and then induced to produce cellulase by removing the xylose and providing only cellulose. A second approach is to improve the environment and cell density of the fungi by using fungi immobilized in a fluidized bed system (CSU).

Another approach to improving enzyme production is to modify the organism. Researchers at Lehigh University are carrying out mutation selection procedures to create mutants that can grow and produce enzyme on soluble sugars such as glucose or xylose, as well as mutants with higher specific or β -glucosidase activities. Researchers at Rutgers University will try to identify the mechanism that regulates cellulase production within the fungi, so that cellulase production can be triggered independent of the external environment.

Xylose Fermentation Research

Five-carbon sugars (primarily xylose) are 15%-30% of the feedstock in agricultural residues and herbaceous crops. Xylose is amorphous, readily hydrolyzed, and can easily be produced by virtually all processes. However, standard industrial yeasts such as *S. cerevisiae* cannot ferment xylose to ethanol. Although several bacteria are available to ferment xylose to higher alcohols, our research program focuses on producing ethanol from the xylose fraction. The five major approaches use alternate yeasts, bacteria, fungi, extracellular xylose isomerase, or genetically altered industrial yeasts. Although none of these routes has yet achieved the rate or ethanol tolerance of the standard six-carbon sugar fermentation, at least one is now reaching the point where it is showing commercial promise.

An analysis recently performed at SERI showed that the most promising means of integrating xylose fermentation with SSF is to separately ferment the xylose containing liquids from pretreatment, and then use this dilute ethanol feed as the dilution water for SSF. This has the advantage of minimizing water use by the process, as well as minimizing the problems of contamination in the SSF reactor. It was shown that if the xylose fermenting organism can only produce ethanol concentrations on the order of 1%, xylose fermentations are actually harmful to the process economics. However, ethanol tolerance beyond 2.5%-3.0% is not necessary. The maximum benefit achievable by xylose fermentation was estimated to be a 25% reduction in ethanol selling price.

When the need for xylose fermentation became apparent, several groups began screening programs to search for xylose-fermenting yeasts. Several such yeasts were identified, the most notable being *Pachysolen tannophilus*, *Pichia stipidis*, and *Candida shehatae*. Optimization of culture conditions and other relatively simple steps have greatly improved the performance of these fermentations in the past few years. Research being performed by TVA shows that ethanol concentrations of 2.0%-2.5% have been achieved with yields 70% of theoretical. These are promising results, and represent the best of any of the xylose fermentations to date.

Bacteria are capable of fermenting xylose to ethanol and other alcohols. *Clostridium thermosaccharolyticum* has been studied at the Massachusetts Institute of Technology (MIT) for more than six years. Several of the mutants can produce ethanol concentrations of 2%. A major drawback is the reduction in yield from undesirable by-products such as lactic and acetic acid. The program is not performing research on bacterial fermentations at this time.

Fungi such as *Fusarium*, which is being studied at Argonne National Laboratory, and the strain NF1, which was isolated at SERI, also can ferment xylose. *Fusarium* fermentation yields are high at low ethanol concentrations, but the rates are low and yield is reduced when ethanol concentrations exceed 1%.

A fourth approach is to use cloning techniques to overproduce the enzyme xylose isomerase in *E. coli*. This enzyme catalyzes the isomerization of xylose to xylulose, another five-carbon sugar, which can be fermented by standard yeasts. Overexpression has been achieved at SERI, and current efforts are aimed at determining the optimal conditions and processes for its use (Lastick et al. 1986). Researchers at Virginia Polytechnic Institute are studying means of producing the enzyme rapidly while maintaining the stability of the plasmid that codes for production of the xylose isomerase enzyme. Researchers at Purdue University are using genetic engineering techniques to improve the production of the enzyme xylulokinase in yeast. Xylulokinase activity is thought to be the key to rapid fermentation of the xylulose to ethanol.

Lignin Utilization Research

Lignin, a phenolic polymer, cannot be fermented to ethanol. With present technology, the lignin is assumed to be burned, producing energy to run the conversion process. However, as with xylose, conversion to a higher value fuel or chemical intermediate could greatly improve process economics. Current research at SERI, in cooperation with many other laboratories, is directed at understanding the effects of various pretreatment and lignin extraction techniques on the structure and potential uses of the lignin fraction, and at converting the lignin into a liquid transportation fuel and octane enhancer (methyl aryl ethers).

These compounds are excellent nonmetallic gasoline-blending agents and octane enhancers. The production process hydrotreats slurried lignin in the presence of a catalyst to produce a mixture of phenolic compounds and hydrocarbons. This mixture could be subsequently thermally dealkylated to produce phenol and benzene (Hydrocarbon Research, Inc. Lignol process). Although phenols are good octane enhancers, they are not suitable for addition to gasoline because of their corrosive nature. The conversion of the phenols into the corresponding methyl aryl ethers avoids the corrosion problems and also the need to add a thermal dealkylation step to strip alkyl groups from the aromatic rings. A process to convert lignins into mixtures of monomeric phenolic compounds and hydrocarbons and to convert the phenolic portion into the methyl aryl ethers is being investigated at SERI. Both the liquid hydrocarbons and the methyl aryl ethers are high-value

octane enhancers. Methyl aryl ethers prepared from coal liquids were tested at Gulf Oil Company and found to be fully compatible gasoline octane enhancers. Efforts are currently focused on identifying and testing catalysts for converting the low-molecular-weight lignins to the mixture of phenols and hydrocarbons, and on assessing the relationship of the lignin feedstock structure on the final product slate. This effort is supported by catalyst development and engineering efforts at the Colorado School of Mines.

Conclusions

Substantial progress has been made in all phases of research in the past several years. In all cases, the keys to improvement are increased yield (use of all fractions of the feedstock, and improved yields in the conversion of each fraction), increased concentrations of the ethanol product, and decreased catalyst costs (Figure 10). In the late 1970s, the predicted cost of ethanol production by enzymatic hydrolysis was \$3.60/gal. With current performance it has been reduced to less than \$2.00/gal, and has the potential to be reduced to under \$1.00/gal.

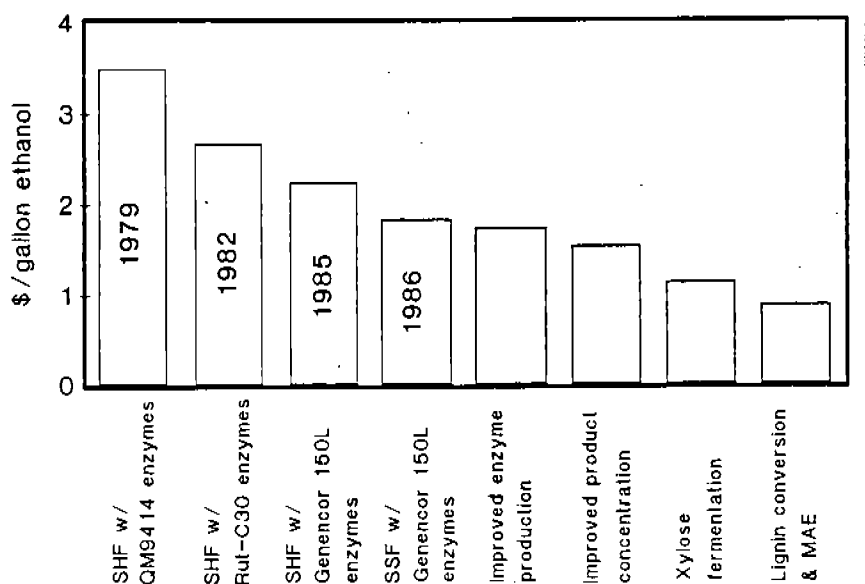


Figure 10. Improvements in Enzymatic Hydrolysis

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